International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 122-127
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF TENOFOVIR DISOPROXIL FUMARATE

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ABSTRACT

A stability indicating assay method was developed and validated according to the ICH guidelines for estimation of Tenofovir Disoproxil Fumarate. HiQSil C18 HPLC column was used. Mobile phase consisting of potassium di hydrogen ortho phosphate buffer of pH -3 : Acetonitrile (70:30 v/v) at a wavelength of 260 nm. Drug was subjected to forced hydrolytic, oxidative, photolytic and thermal degradation conditions. Degradation was observed for Tenofovir Disoproxil Fumarate in hydrolytic conditions.

KEY WORDS

Tenofovir Disoproxil Fumarate, Stress degradation, Validation, Stability Indicating.

INTRODUCTION

Tenofovir Disoproxil Fumarate (TDF) chemically is 9-[(R)-2[[bis[[(isopropoxycarbonyl)Oxy] methoxy] phosphinyl] methoxy] propyl] adenine fumarate [1]. A nucleotide reverse transcriptase inhibitor (NtRTI), the ester prodrug of tenofovir which is hydrolyzed to tenofovir intracellularly and phosphorylated to the active metabolite, tenofovir diphosphate. Tenofovir is a nucleotide analogue of deoxyadenosine monophosphate, with activity against HIV-1, HIV-2 and Hepatitis B virus (HBV). Literature survey revealed that there are number of stability indicating HPLC methods [2-7] reported but the results of stress degradation do not match, hence it was thought necessary to confirm the same by developing Stability Indicating HPLC method.

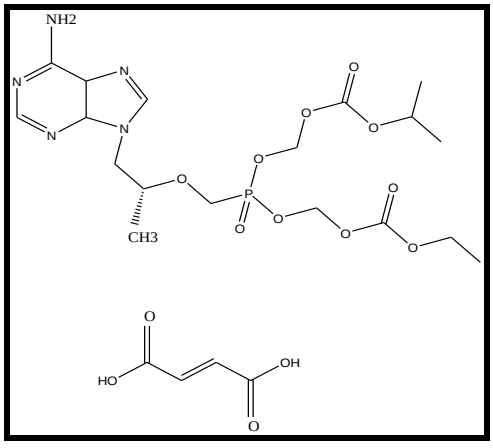

Fig 1: Chemical structure of Tenofovir Disoproxil Fumarate

MATERIALS AND METHODS

Chemicals and Reagents

The standard drug Tenofovir Disoproxil Fumarate was procured as gift sample from Mylan Pharmaceuticals Pvt Ltd. Other chemicals purchased were Methanol from LOBA Chemie
(Mumbai) and Acetonitrile (HPLC grade) from S D Fine – chem Limited (Mumbai) and potassium di hydrogen ortho phosphate LOBA Chemie. Mumbai. O-phosphoric acid Loba chemicals, Mumbai were used for the study. Water (HPLC grade) generated by through PURELAB UHQ (ELGA system).

INSTRUMENTS

HPLC

JASCO-PU 2080 PLUS intelligent HPLC pump.
MD 2010 PDA detector
Borwin- PDA software (version 1.5)
20 µl Rheodyne injector port.
Hamilton Syringe (100 µl)

Optimised Chromatographic Conditions:

The chromatographic system consisted of JASCO-PU 2080 PLUS intelligent HPLC pump with PDA detector. The software used was Borwin software. A 20 µl Rheodyne injector port was used for injecting sample solutions. A HiQSil C_{18}HS (250×4.6mm; 5 µm particle size) column was used for the analysis. The mobile phase consisted of 10 mM Potassium Dihydrogen Phosphate and Acetonitrile (70:30v/v). The pH of aqueous part was adjusted with o-phosphoric acid to (pH 3). The mobile phase was filtered through 0.45 µm membrane filter followed by sonication for 10 min using bath sonicator. The optimum wavelength selected for quantification was 260 nm with a total run time of 10 min. Peak of Tenofovir Disoproxil Fumarate was eluted at 3.907 ± 2 mins.

Preparation of solutions

Preparation of standard stock solution of drugs

Standard stock solution of drug Tenofovir Disoproxil Fumarate of concentration of 1000 µg/ml was prepared in methanol by dissolving 10 mg of drug in 10 ml of methanol. The standard stock solution was diluted with the mobile phase to get solution in concentration ranging from 5 µg/ml to 30 µg/ml.

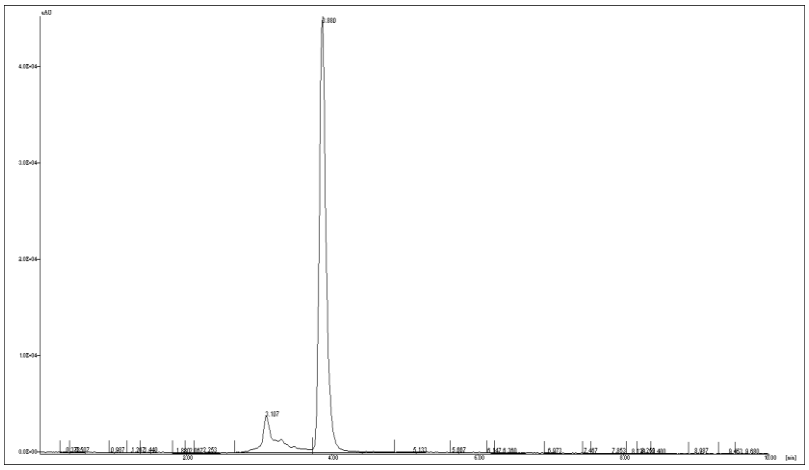

Fig 2. Standard chromatogram of Tenofovir Disoproxil Fumarate

Table 1: System suitability of 10 ug/ml Tenofovir Disoproxil Fumarate chromatogram

Sr.No.	Conc (ug/ml)	Rt (min)	Area	Plates	Resolution	Asymmetry
a)	10 ug/ml	3.90	162561.30	9468.62	4.22	1.32

FORCED DEGRADATION STUDIES [8]

To provide an indication of the stability-indicating ability and specificity of the proposed method forced degradation studies were performed. Hydrolysis under acidic, alkaline & neutral & oxidative & thermal and photolytic degradation studies were conducted on Tenofovir Disoproxil Fumarate.

Acid degradation:

TDF working standard was weighed accurately & dissolved in MeOH & volume was made up to 10ug/ml & it was further diluted to make 100 µg/ml solution.

From that 1.5 ml of sample was pippeted out. 1ml of 0.1 N HCL was added. Volume was made up & sample aliquots was kept for overnight degradation & examined. Sample was neutralized using 0.1 N NaOH before injection.

Alkaline degradation:

From stock solution of 100 ug/ml 1.5 ml of sample was pippeted out. 1ml of 0.1 N NaOH was added. Volume was made up & sample aliquots were kept for overnight degradation & examined. Sample was neutralized using 0.1 N HCL before injection.

Oxidative degradation

From stock solution of 100 ug/ml 1.5 ml of sample was pippeted out. 1ml of 0.6% H_2O_2 was added. Volume was made up & sample aliquots were kept for overnight degradation & examined.

Neutral degradation

From stock solution of 100 ug/ml 1.5 ml of sample was pippeted out. 1ml of distilled Water was added. Volume was made up & sample aliquots were kept for overnight degradation & examined.

Thermal Degradation

Tenofovir Disoproxil Fumarate was weighed accurately & exposed to 80 ^{0}C for 8 hrs. It was cooled to room temperature, weighed, dissolved in methanol & diluted to 100 ug/ml solution. From that 1.5 ml sample was pipetted out & volume was made up with methanol & examined.

Photolytic degradation

Tenofovir Disoproxil Fumarate was exposed to UV light for (200-watt hours/square meter) and to fluroscent light (1.2million Lux hours). The samples were allowed to cool. 10 mg of standard Tenofovir Disoproxil Fumarate was weighed and volume was made up. Further diluted with mobile phase to attain the concentration of 15 µg/ml.

Table 2. Forced degradation studies of Tenofovir Disoproxil Fumarate

Sr.No.	Degradation condition	% Recovery	Purity Tail	Purity front
1.	**Acid Hydrolysis** (RT 24hrs) (0.1 N HCl)	71.20	999.41	999.89
2.	**Base Hydrolysis** (RT 24hrs) (0.1 N NaOH)	84.99	999.59	999.33
3.	**Oxidation** (RT 24hrs) (0.6 % H_2O_2)	68.18	998.0	999.70
4.	**Neutral** (RT 6 hrs Distilled water)	83.20	997.06	998.60
5.	**Photolytic Degradation** (Exposure to UV light for 200-watt hours)	62.88	999.05	999.06
6.	**Fluroscence Degradation** **(**Exposure to light for 1.2 million lux hours)	65.18	979.19	999.10
7.	**Thermal degradation** **(**Exposure to dry heat at 80ºC for 6 Hrs)	73.81	998.02	998.05

METHOD VALIDATION [9]

The method was validated in terms of linearity, range, precision, accuracy, specificity, limit of detection, limit of quantitation and robustness as per ICH guidelines.

Specificity:

The specificity of the method was checked by peak purity profiling studies of stress degradation samples. The peak purity values was found to be more than 990. This indicates there is non-interference of any other impurity or degradation product. Thus, the method is said to be specific.

Linearity & range

Six different concentrations of drug (5, 10, 15, 20, 25 and 30 µg/ml) were prepared from standard stock solution of 1000 µg/mL of Tenofovir Disoproxil Fumarate and was analysed. The peak areas were plotted against the corresponding concentrations to obtain a linearity plot. The linear regression equation and correlation co-efficient (r^2)

obtained for Tenofovir Disoproxil Fumarate was y= 26630 x + 35100 and r^2 = 0.983 respectively.

Precision:

The precision was evaluated with respect to both repeatability and intermediate precision. Repeatability was evaluated by injecting six replicate injections of test solution of the drug. Tenofovir Disoproxil Fumarate (10 µg/ml). The studies were repeated for three different days to determine intermediate precision. Peak areas of the drugs were determined and % RSD was calculated.

Table 3: Precision of Tenofovir Disoproxil Fumarate

INTERDAY PRECISION					
Sr. No.	**Method**	**Amount conc ug/ml**	**Mean**	**SD**	**% RSD**
1.	HPLC	5 ug/ml	141420.3	1538.715	1.08
INTRADAY PRECISION					
1.	HPLC	5 ug/ml	139303.6	1888.782	1.35

Assay:

Assay of Tenofovir Disoproxil Fumarate was done by spike blend method. 300 mg of Tenofovir Disoproxil Fumarate was mixed with equal amounts of starch and lactose to make 500 mg of spike blend. The contents of spike blend were properly mixed. Powder equivalent to 10 mg of Tenofovir Disoproxil Fumarate was accurately weighed and transferred into a 10 ml volumetric flask and volume was made up with methanol. The volumetric flask was sonicated for 10 min to enable complete dissolution of Tenofovir Disoproxil Fumarate and the solution was filtered through whatmann filter paper. From the filtrate further dilution was made with methanol to get 100 µg/ml solution. Finally, this solution was further diluted with mobile phase to yield a concentration of 10 µg/ml and then it is injected. The results obtained for assay of Tenofovir Disoproxil Fumarate are shown in Table.

Table 4: Assay of Tenofovir Disoproxil Fumarate

Peak area (avg)	Amount Recovered (µg/ml)	% Recovery	Mean % Recovery	SD	% RSD
307264.9	10.23	102.30	102.20	0.69	0.68

Accuracy:

The accuracy of the method was assessed by the recovery studies at three different concentrations by the addition of known amount of standard to the test blank blend. The drugs were spiked at three different levels i.e., 80 %, 100% & 120 %. The recovery was calculated by slope and intercept of the linearity plot of drugs. The results obtained for accuracy are presented in the Table.

Table 5: Accuracy for Tenofovir Disoproxil Fumarate

Level of % accuracy	% Recovery
80 %	92.54
100 %	102.91
120 %	97.94

Limit of detection (LOD) and Limit of Quantitation (LOQ):

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S where, σ = standard deviation of the y-intercept of linearity equations and S = slope of the calibration curve of the analyte. LOD was found to be 0.56 µg/ml. LOQ was found to be 1.72 µg/ml.

Robustness:

Robustness was evaluated by deliberate variation in parameters like ratio of mobile phase components, pH and concentration of buffer. The pH of the mobile phase was varied and concentration was varied. Robustness was studied at a concentration of 10µg/ml for Tenofovir Disoproxil Fumarate. The results were found to be unaffected by small changes in pH of mobile phase and concentration of buffer.

Table 6: Summary of Validation Studies:

Sr.no.	Validation Parameters		Tenofovir Disoproxil Fumarate
1.	**Specificity**		Specific
2.	**Linearity**		y= 26630 x + 35100 r^2 = 0.983
	Range		(5-30 ug/ml)
3.	**Precision**	**Interday**	1.08%
		Intraday	1.35%
4.	**Assay**		102.20 %
5.	**Recovery**	**80 %**	92.45 %
		100 %	102.91 %
		120 %	97.94 %
6.	**Limit of Detection (LOD)**		0.56 µg/ml
7.	**Limit of Quantitation (LOQ)**		1.72 µg/ml
8.	**Robustness**		Robust

CONCLUSION:

TDF showed degradation in acidic medium (0.1 N HCl) and in alkaline medium (0.1 N NaOH) at room temperature. It also showed degradation under neutral hydrolytic, oxidative, neutral, photolytic and thermal degradation conditions. The % degradation was calculated by comparing the peak areas of standard drug with peak areas of drug under degradation conditions. We conclude that the proposed method is stability indicating since the drug peak showed no interference as proved by peak purity and can be used for estimation of TDF.

ACKNOWLEDGEMENT:

Authors are thankful to the Principal and the management of AISSMS College of Pharmacy, Pune, for providing the necessary facilities for research work & Mylan Lab, Pvt. Ltd, for providing free gift sample of Tenofovir Disoproxil Fumarate.

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Received:02.05.18, Accepted: 05.06.18, Published:01.07.2018

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